Pesquisa Florestal Brasileira
Brazilian Journal of Forestry Research
www.cnpf.embrapa.br/pfb

Mineral content of young leaves of yerba mate

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Termos para indexação:
Ilex paraguariensis
Bebida
Recomendações nutricionais

Index terms:
Ilex paraguariensis
Bevarages
Nutritional recommendations

Histórico do artigo:
Recebido em 20/09/2013
Aprovado em 04/03/2014
Publicado em 04/04/2014

doi: 10.4336/2014.pfb.34.77.594

Resumo - Yerba mate is largely used to produce drinks to human consume. In Spring the plants develop tender young leaves that could represent a new market niche in the yerba-mate industry; this period is called as ”pressafrinha” in this study. This study aimed to analyze the total and hydro soluble nutritional values of young leaves collected in the Spring season and its nutritional potential for human consumption. To represent the ”pressafrinha” young leaves, the collected vegetal material was limited to the third terminal bud. The total and hydro soluble chemical analysis of leaves in the provenances Cascavel (progeny 174), Ivaí (progeny 6), Barão de Cotegipe (progenies 68 and 69) included the following elements: P, K, Ca, Mg, Na, Fe, Mn, Cu and Zn. The content of elements in the beverage obtained from the infusion of young leaves of yerba mate in the recommended daily intake ranges from 0.5% to 11.5% and was in the following order: Cu > Mn > K > P > Mg > Fe > Zn > Ca > Na. Progenies and morphotypes of yerba mate showed little variation in the total nutrient content and did not differ in the content of soluble nutrients evaluated in young leaves. The extract obtained from young leaves of yerba mate presents potential to be used for human consumption.

Valor nutricional de folhas jovens de erva-mate

Abstract - A Erva-mate é muito usada para produção de bebidas para consumo humano. Na primavera, a planta desenvolve folhas jovens que podem representar um novo nicho de mercado na indústria de erva-mate; este período é chamado nesse estudo de prea-safrinha. Este estudo teve por objetivo investigar os teores nutricionais totais e hidrossolúveis de folhas jovens de erva-mate coletadas na primavera e seu potencial nutricional para o consumo humano. Para representar folhas jovens da pre-safrinha, o material vegetal recolhido foi limitado ao terceiro botão terminal. A análise química total e hidrossolúvel de folhas das procedências Cascavel (progênie 174), Ivaí (progênie 6), Barão de Cotegipe (progênies 68 e 69) incluiu os seguintes elementos: P, K, Ca, Mg, Na, Fe, Mn, Cu e Zn. O conteúdo dos elementos da bebida obtida a partir da infusão de folhas jovens de erva-mate na ingestão diária recomendada variou de 0,5 a 11,5 % e se apresentou na seguinte ordem: Cu > Mn > K > P > Mg > Fe > Zn > Ca > Na. Progênies e morfotipos de erva-mate mostraram pouca variação no conteúdo total de nutrientes e não diferiram quanto ao conteúdo de nutrientes hidrossolúveis avaliados nas folhas jovens. Sendo assim, o extrato obtido a partir de folhas jovens de erva-mate apresenta potencial de uso para o consumo humano.

Introduction

Essentially south-american, Yerba mate (*Ilex paraguariensis*) naturally occurs in an area of approximately 540,000 km², distributed in Brazil, Argentina and Paraguay (Rotta & Oliveira, 2010). Due to its large geographical distribution, morphological variation were observed in the leaves (Coelho et al., 2002), which can be denominated as morphotype (Reissmann et al., 2003; Dünish et al., 2004). The Amarelinha morphotype is characterized by leaves with a light hue at the limb region and at the main and secondary rib by a yellowish hue; Cinza morphotype presents leaves with a greenish-gray rib and the main rib is not yellowish as in Amarelinha morphotype; and Sassafrás morphotype has leaves with a glossy dark green limb on its adaxial side and ribs with a lighter color (Reissmann et al., 2003). The branches and leaves from yerba mate are used to prepare teas and the regional drink “chimarrão”. Furthermore, it is used in inks, disinfectants and as the primary source of some medications (Pandolfo et al., 2003). The main harvest of yerba mate is called "safra" and occurs between May and September. The second harvest, known as "safrinha", occurs between December and February (Andrade, 2003). During the Spring, between these two harvests periods, the plant develops tender young leaves that might represent a new market niche in the yerba mate industry; this period is called "pre-safrinha" in this study. A harvest of young leaves would enable the producer to obtain a new product, adding income in a distinct period from "safra" and "safrinha".

The infusion of young leaves, with species such as *Camellia sinensis*, *Clerodendrom glandulosum* and *Saraca asoca*, is an usual practice in indian tribes due its medicinal properties (Nath et al., 2011). In addition, studies using other species have attempted to analyse the chemical characteristics of young leaves consumed as tea, to verify its potential use in human health and nutrition (Chenery, 1995; Fung et al., 2003; Fung et al., 2009; Shu et al., 2003; Han et al., 2007; Xie et al., 2007; Shi et al., 2008). This study aimed to analyze the total and hydrosoluble nutritional values of young leaves of yerba mate collected in the Spring and its nutritional potential for human consumption.

Material and methods

The experiment was carried out in 1997, in the municipalty of Pinhais, Paraná State, Brazil, in the Experimental Station, from the Federal University of Paraná. The climate of the region is classified as Cfb in the Köppen classification, with a mean annual minimum temperature of 16.2 °C and a maximum of 25.9 °C, and a mean annual precipitation of 2,376 mm (Simepar, 2012). The experimental soil shows an open clay texture and medium to high fertility (Table 1).

The experimental model used was a 8 × 8 lattice (64 families, 52 from Paraná state, municipality of Colombo, São Mateus do Sul, Ivaí and Cascavel, and 12 from the municipality of Barão de Cotegipe, Rio Grande do Sul State), with nine balanced repetitions and six plants per plot (54 plants per progeny), totaling 3,456 plants.

Two morphotypes were chosen for the present study: Sassafrás and Amarelinha, due to their use in other studies (Kishi, 2001; Borrille et al., 2005; Robassa, 2005; Oliva, 2007; Guimarães et al., 2010). Amarelinha morphotype was represented by progeny 174 (derived/provenance from Cascavel – Paraná State) and 6 (provenance from Ivaí – Paraná State), and Sassafrás

Table 1. Chemical properties and soil texture, at 0-–0.2 m depth with yerba mate (*Ilex paraguariensis*) grown for 16 years (Paraná-Brasil, 2011).

pH	Texture: Sand	Silt	Clay	C	P	Micronutrients: Fe	Mn	Cu	Zn
	g kg^{-1}			g dm^{-3}	mg dm^{-3}	mg kg^{-1}			
4.43	440	200	360	2.83	8.93	76.38	11.97	1.03	0.8

Exchangeable bases: Ca^{2+}	Mg^{2+}	K^+	Na^+	Sum of bases	Extractable acidity: H^+	Al^{3+}	Base saturation	Aluminum saturation
$cmol_c$ dm^{-3}							%	%
3.7	2.9	0.2	0.2	7.0	8.7	1.28	70	15.46

morphotype, by progenies 68 and 69 (provenance from Barão de Cotegipe – Rio Grande do Sul State).For each progeny, six trees were collected, representing their repetition, totalizing 24 trees analyzed.

Material was sampled in plot 6, due to morphological and phytosanitary aspects of the plants. Because of this, the experimental design used was random blocks, with six repetitions, each repetition being represented by one half-sister tree.

In November 2011, representing the Spring season, young leaves limited to the third terminal bud were harvested. Aiming at leaves with a maximum light exposure, young leaves were collected from the medium portion of the live canopy, with a northerly exposure (Zöttl, 1987; Jones Junior & Case, 1990).

The branches were separated from the leaves in the laboratory and 100 leaves were counted to obtain the fresh mass. Subsequently, the leaves were washed with deionized water and dried in a kiln at 60 ºC to constant weight. The same leaves were then reweighed to obtain the dry mass. The leaves were milled, sieved to 1 mm and packed in jars protected from light.

For the chemical analysis of the total leaf contents of P, K, Ca, Mg, Na, Fe, Mn, Cu and Zn, 1 g plant material was weighed in porcelain crucibles for incineration in a muffle furnace at 500 °C and subsequently digested with acid (3 mol L^{-1} HCl). The P content was determined by colorimetry via ammonium molybdate-vanadate with a UV-VIS I spectrophotometer (Shimadzu - UV mini 1240). The determination of K and Na was performed by photometry emission (Digimed-DM 62), and of Ca, Mg, Fe, Mn, Cu and Zn by atomic absorption spectrophotometry with flame (Varian-AA240FS) (Martins & Reissmann, 2007).

The extract for determination of soluble nutrients was obtained by infusion of 3 g milled and sieved sample in 60 mL of deionized water heated to 80 °C (± 5 °C). The extract was kept warm on a hotplate for five min with subsequent filtration through a blue-band paper filter 3893 (adapted from Reissmann et al., 1994). A 10 mL aliquot of the filtered extract was transferred to a crucible, to be evaporated. After evaporation, the digestion and determination of macro-and micronutrients were performed as previously described.

Based on the concentration of hydrosoluble nutrients, the contribution of yerba mate to the recommended daily intake (RDI) was determined. The RDI refers to the amount of vitamins and minerals that should be consumed daily to meet the nutritional needs of most individuals and groups of people in a healthy population.

The data were submitted to analysis of variance (ANOVA) and mean comparison test (Tukey 1% and 5% of significance). The soluble values of copper were log-transformed for analysis.

Results and discussion

Vegetative biomass

The mean fresh mass was not statistically different among the progenies (Figure 1). However, the mean dry mass was greater for provenance 6 (14.85 g 100 $leaves^{-1}$) and 174 (14.30 g 100 $leaves^{-1}$), both of Amarelinha morphotype, compared to that of provenance 68 (10.66 g 100 $leaves^{-1}$) and 69 (10.64 g 100 $leaves^{-1}$) of Sassafrás morphotype. This difference of approximately 36% shows that Amarelinha morphotype has a higher potential for dry matter production than Sassafrás.

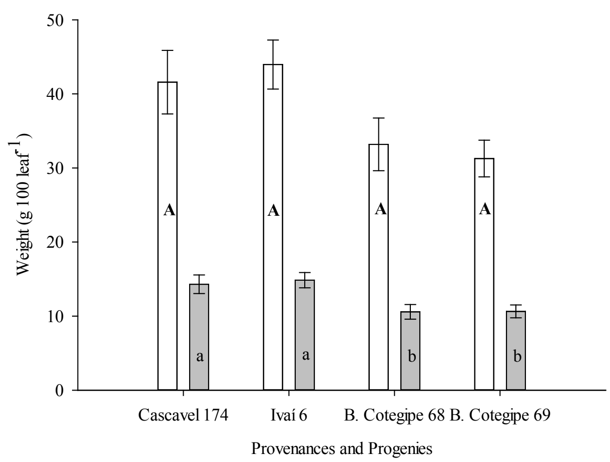

Figure 1. Fresh mass (white) and dry mass (gray) of yerba mate young leaves in four progenies collected in Spring (means with the same capital letter or lower case letter did not differ statistically in the mass of fresh and dry matter, respectively (Tukey $p > 0.05$).

Oliva (2007) found mean dry mass values of leaves harvest at "safrinha" 35% higher than those obtained in this study. These results reflect differences of dry mass when harvesting young leaves. So, it should be necessary to adapt the management and the industrial process to use this new raw material.

Leaf nutritional values and participation in the recommended daily intake

In this study, the RDI was based on nutritional values for adults and estimated according to Castellsangué et al. (2000), which considers the mean daily consumption of yerba mate infusion in the South-American region of 0.9 L per person.

The total hydrosoluble nutrition values of young leaves (Tables 2 and 3), were lower than those observed in mature leaves found by Malik (2008) to Ca (12.72 g kg^{-1}), Mg (8.11 g kg^{-1}), Mn (816 mg kg^{-1}), Fe (85.5 mg kg^{-1}), Zn (30.5 g kg^{-1}) and Cu (12.7 g kg^{-1}), similar to P (1.39 g kg^{-1})and superior for K (12.66 g kg^{-1}).

The available total and hydrosoluble value of P did not differ between the studied progenies (Table 2) and mean total values were close to those observed in young leaves of *Camellia sinensis* (1.48 g kg^{-1}) collected from 14-year-old plants (Carr et al., 2003). They were, however, lower than those found on green tea leaves (3.66 g kg^{-1}), white tea (6 g kg^{-1}) and flower heads of chamomile (5.99 g kg^{-1}) (Malik et al., 2008). The mean hydrosolubility ranged between 0.2 and 0.5 g kg^{-1}, being these values lower than the ones found in the grean tea leaves extract (1.35 g kg^{-1}), white tea (1.11 g kg^{-1}), flower heads of chamomile (3.08 g kg^{-1}) and mature leaves of yerba mate (1,01 g kg^{-1}) (Malik et al., 2008).

Due to the use of P in plants as a structural component of macromolecules and as a prominent component of nucleic acids (Marschner, 2012), it is believed that low amounts can be hydrosolubilized. However, the Spring period is responsible for a high redistribution of P in plants (Marschner, 2012), which might have affected the quantity of extracted P. These quantities, when compared to human requirements, ensure the third greatest participation in the RDI despite being the second element required (Table 4).

Total concentrations of K were statistically different, with the progeny 69 having higher values than progeny

Table 2. Total, hydrosoluble and percentage hydrosolubility values of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in young leaves of Yerba mate (*Ilex paraguariensis*) collected in the Spring season (Paraná State, Brazil, 2011).

Provenance	Progeny	P	K	Ca	Mg
		Total value			
		------------------ g kg^{-1} ------------------			
B. Cotegipe	68	2.1 a[1]	12.9 b	2.2 ab	4.1 a
B. Cotegipe	69	1.8 a	19.0 a	1.5 b	2.7 b
Ivaí	6	2.3 a	17.3 ab	2.2 ab	3.8 a
Cascavel	174	1.8 a	15.7 ab	2.7 a	4.4 a
Average		2.0 a	16.2	2.2	3.8
		Hydrosoluble value			
		------------------ g kg^{-1} ------------------			
B. Cotegipe	68	0.4 a	9.5 a	0.1 a	0.2 a
B. Cotegipe	69	0.3 a	12.7 a	0.1 a	0.1 a
Ivaí	6	0.3 a	9.9 a	0.1 a	0.1 a
Cascavel	174	0.4 a	12.9 a	0.1 a	0.1 a
Average		0.3	11.2	0.1	0.1
		Hydrosolubility percentage			
		------------------ % ------------------			
B. Cotegipe	68	22.2	84.2	5.1	4.5
B. Cotegipe	69	19.2	66.0	9.2	4.6
Ivaí	6	15.0	57.5	4.4	2.4
Cascavel	174	28.5	80.0	3.4	4.2
Average		21.2	72.0	5.5	3.9

Means with different letter differ statistically for total (Tukey $p < 0.01$) or the hydrosoluble values (Tukey $p < 0.05$) for each element. Means with different letter were statistically different for each provenance and each nutrient (Tukey $p < 0.01$) and for the hydrosoluble value (Tukey $p < 0.05$).

68, both coming from Barão de Cotegipe (Sassafrás morphotype) (Table 2). The mean total value was close to the obtained in young leaves of *Camellia sinensis* (Carr et al., 2003), lower to what was observed in green tea (18.54 g kg^{-1}) and in flower heads of chamomile (23.48 g kg^{-1}), and superior to the values obtained in white tea (9.48 g kg^{-1}).

The high value of hydrosoluble K (Table 2) is probably because the element is more absorbed as it found predominantly as a free cation, facilitating its displacement in the cells or plant tissues (Lindhauer, 1985), what also results in higher extractable levels with hot water. The World Health Organization (WHO) recommends an increase in potassium intake to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. According to WHO, this value sould be at least 3,510 mg daily, participating in 2.5% of the RDI if the extract obtained by the young leaves were consumed. The assay of K extracted from young leaves was lower when compared to the value found by Malik et al. (2008) on leaves extract of green tea (23.5 g kg^{-1}), white tea (15.5 g kg^{-1}) and on flower heads of chamomile (19.3 g kg^{-1}), and close to the data obtained with mature leaves of yerba mate (12.65 g kg^{-1}).

The total concentration of Ca obtained in this study (Table 2) was lower than that measured in yerba mate leaves collected six months after the complete pruning of trees (Jacques et al., 2007), who obtained values between 4.46 and 4.8 g kg^{-1}. Among progenies, Cascavel 174 progeny (Amarelinha morphotype) had the biggest content compared to the other progenies (Table 2). The mean hydrosoluble Ca content was the fourth lowest among all elements studied, possibly due to its strong involvement with the cell wall and plasma membrane (Marschner, 2012). The low hydrosolublity and difficult extraction of Ca into the beverage and, when associated with the high levels indicated by OMS, result in a lower nutrient participation by Ca in the RDI (Table 4). The

Table 3. Total, hydrosoluble and percentage hydrosolubility values of copper (Cu), manganese (Mn), zincon (Zn), Iiron (Fe) and sodium (Na) in young leaves of Yerba mate (*Ilex paraguariensis*) collected in the Spring season (Paraná State, Brazil, 2011).

Provenance	Progeny	Cu	Mn	Zn	Fe	Na
				Total value		
			------------------ mg kg^{-1} ------------------			
B. Cotegipe	68	7.2 a[1]	190.4 ab	20.1 a	12.1 b	5.7 c
B. Cotegipe	69	6.2 a	286.6 a	23.0 a	35.4 a	11.7 a
Ivaí	6	6.2 a	325.4 a	26.3 a	28.5 a	10.0 ab
Cascavel	174	7.2 a	137.2 b	30.8 a	28.4 a	8.2 bc
Average		6.7	234.9	25.0	26.1	8.9
				Hydrosoluble value		
			------------------ mg kg^{-1} -----------------			
B.Cotegipe	68	2.1 a	3.1 b	1.2 a	4.7 a	1.2 a
B.Cotegipe	69	3.2 a	5.0 a	1.4 a	6.0 a	0.7 a
Ivaí	6	2.0 a	4.1 ab	1.7 a	5.8 a	0.7 a
Cascavel	174	2.5 a	4.4 ab	1.1 a	6.7 a	0.8 a
Average		2.5	4.2	1.4	5.8	0.8
				Hydrosolubility percentage		
			------------------ % ------------------			
B.Cotegipe	68	30.5	1.2	6.6	47.3	24.4
B. Cotegipe	69	40.5	1.9	6.4	17.3	6.4
Ivaí	6	33.7	1.3	5.9	19.9	7.0
Cascavel	174	39.8	3.3	4.2	23.9	12.7
Average		36.1	2.0	5.8	27.1	13.0

Means with different letter differ statistically for total (Tukey $p < 0.01$) or the hydrosoluble values (Tukey $p < 0.05$) for each element. Means with different letter were statistically different for each provenance and each nutrient (Tukey $p < 0.01$) and for the hydrosoluble value (Tukey $p < 0.05$).

low hydrosolubility can be found also on leaves extract of green tea (0.18 g kg^{-1}) and white tea (0.24 g kg^{-1}), however, when compared with the mature leaves of yerba mate (1.97 g kg^{-1}) it was observed that the values in the young leaves are lower by almost 20 times (Malik et al., 2008).

Table 4 . Mean nutritional value of the drink obtained by the infusion of young leaves of four yerba mate (*Ilex paraguariensis*) progenies collected in the Spring season and its recommended daily intake, nutritional content consumed daily and proportion of the recommended daily intake (RDI) (Paraná State, Brazil, 2011) .

Nutrients	Recommended daily intake	Nutritional content consumed daily (900 mL) (*)	Participation in the RDI (%)
P (2)	700 mg	17.0 mg	2.4
K (4)	3510 mg	89.4 mg	2.5
Ca (1)	1000 mg	4.5 mg	0.5
Mg (1)	260 mg	6.0 mg	2.3
Mn (2)	23 mg	0.2 mg	6.5
Fe (1)	14 mg	0.3mg	2.1
Zn (1)	7 mg	0.1mg	0.9
Cu(1)	900 µm	103.5 µm	11.5
Na (3)	2400 mg	0.04 mg	0.001

(1) Human vitamin and mineral requirements, Report 7ª Joint FAO/OMS Expert Consultation Bangkok, Thailand, 2001. (2) Dietary reference intake, Food and Nutrition Board, Institute of Medicine. 1999-2001. (3) Resolução RDC nº 360, de 23 de dezembro de 2003 (Anvisa, 2003). (4) OMS. Guideline: Potassium intake for adults and children (World Health Organization, 2012). (*) Mean daily consumption of yerba mate, for the South American region, defined by Castellsangué et al. (2000).

The total mean Mg, and Ca values were lower than those found in young leaves of yerba (7.62 ± 1.8 g de Mg kg^{-1} and 4.65± g de Ca kg^{-1}) (Jacques et al., 2007), with the lowest value found in Barão de Cotegipe 69 progeny (Sassafrás morphotype) (Table 2). The Mg hydrosoluble percentage (Table 2) was close to that obtained in young leaves of *Camellia sinensis* (Carr et al., 2003), but less than that reported in the literature, which showed a Mg hydrosolubility of 60–90% (Fink, 2012). Studying a variety of teas, Malik et al. (2008) found higher percentages of hydrosolubility of Mg as, for example, 73% on white tea leaves and 63% on mature leaves of yerba mate. Despite being the element with the third-highest nutritional requirement , the low hydrosoluble levels of Mg in yerba mate gave it the fourth place in the RDI among the elements studied (Table 4).

The total and hydrosoluble values of Cu and Zn did not differ statistically between the provenances (Table 3) and were close to the values obtained in young leaves of yerba mate by Jacques et al. (2007). Although Cu is known to be an element with low mobility, because it is present in chloroplasts or enzyme-linked (Marschner, 2012), high levels were found in soluble extracts obtained from yerba mate infusion (Castellsangué et al., 2000) and in other infusions from plants such as chamomile (Basges & Erdemoglu, 2006).This high level of soluble Cu in hot water makes the yerba mate infusion contributes with approximately 10% of the RDI, the largest contribution compared to the other elements (Table 4) and it is also the element with the quantities recommended amounts. The hydrosolubility of Zn was low (Table 3), possibly due to its participation in metabolic functions and its ability to bind to nitrogen, oxygen and sulfur, acting in enzymatic reactions in functional and structural forms (Valee & Auld, 1990) . The recommended daily amounts of Zn for adults are small. However, due to its low hydrosolubility, its participation in RDI was low, resulting in it being the nutrient with the second lowest participation in the RDI (Table 4).

The total value of Mn in the progenies Ivaí 6 (Amarelinha morphotype) and Barão de Cotegipe 69 (Sassafrás morphotype) were higher than that in progeny Cascavel 174 (Amarelinha morphotype), and the hydrosoluble value of progeny Barão de Cotegipe 69 was higher than that of Barão de Cotegipe 68 (Table 3). Mn is considered a nutrient that can be easily extracted from leaves (Camargo & Silva, 1975). So,the mean hydrosoluble percentage of Mn was much lower than expected (2%). Even though the amounts of Mn observed in the leaves of yerba mate were relatively high and as the daily recommendation of Mn is small when compared to the other elements, it was the second highest component of the RDI (Table 4). Extracts obtained from green tea leaves (130.5 mg kg^{-1}) and white tea (130.5 mg kg^{-1}) studied by Malik et al. (2008) presented Mn values higher than those obtained from young leaves of yerba mate.

Fe content differed significantly among the progenies, with the lowest mean total value obtained in the progeny Barão de Cotegipe 68 (Sassafrás morphotype) (Table 3). The mean hydrosoluble percentage of Fe was 27% and contrasts with values cited in the literature, where low hydrosolubility was attributed to the strong links of the element with the organic structures of plants, especially to Fe enzymes (Marschner, 2012). Plants as green tea (12.12 mg kg^{-1}), white tea (130.5 mg kg^{-1}) and mature leaves of yerba mate (3.95 mg kg^{-1}) present the lowest levels found in young leaves, showing a greater water soluble potential of Fe in tissue of young species. These low soluble Fe levels gave the element the third lowest nutritional participation in the RDI (Table 4).

The mean total Na values were higher in the progenies Ivaí 6 and Barão de Cotegipe 69 (Table 3). Na is normally absorbed in its ionic form (Na^+) and it is relatively mobile in plants, with some functions similar to K (Korndöfer, 2006). However, its hydrosolubility was lower than expected. A Brazilian food guidance recommends that daily sodium intake does not exceed 1.7 g (Brasil, 2005), and Resolution RDC No. 360 recommends levels not higher than 2.4 g. The levels found in the drink are approximately 100 times lower than recommended. The low levels of sodium demonstrate that consumers of the young leaves should not develop problems such high blood pressure, stroke risk, left ventricular hypertrophy and a progression of renal disease (Suen et al., 2013), arising from the daily consumption of the proposed tea due to the low Na concentration.

This study was a preliminary attempt to justify the viability of introducing young leaves into the yerba mate market. The contribution of tea obtained by the infusion of young leaves to the RDI is lower than levels found in adult leaves that are currently in the market, for almost all elements with the exception of P and K, which are present in higher concentrations than those reported in the literature. In despite of this minor contribution, the nutritional content of this new kind of tea is favorable to adults in terms of daily nutrient intake. Research with these young leaves should be continued, to evaluate their organoleptic and nutritional characteristics as well as the market acceptance and economic viability.

Conclusions

The content of elements in the extract (beverage) obtained from the infusion of young leaves of yerba mate in the recommended daily intake ranges from 0.5% to 11.5% and the nutrient content was in the following order: Cu > Mn > K > P > Mg > Fe > Zn > Ca>Na.

Progenies and morphotypes of young Yerba mate leaves did not differ in the levels of soluble nutrients.

The nutrients levels are within the limits required for the recommended daily intake, therefore, the extract obtained from young leaves of yerba mate has nutritional potential for human consumption.

Acknowledgement

Embrapa Florestas, CNPq and UFPR

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